iii) Determination of AChR content

[00130] AChR content was determined by measuring α-bungarotoxin (α-BTX) binding sites. Tested samples were derived from (a) muscle preparations or from (b) cells grown in a tissue culture.

[00131] a) For the muscle preparation, the procedure described by Souroujon et al. (1985) was essentially followed. Briefly, muscle tissue was removed and homogenized in a Sorvall omnimixer for 2 min. at full speed. Two volumes of Tris-HCl buffer, pH 7.5, containing 0.1 M NaCl, 1 mM EDTA, 0.1 mM PMSF and 0.5 mM NaN_3, were used for homogenization. Homogenates were then centrifuged at 48,000 x g for 1 h, washed once and recentrifuged as above. The homogenates were stirred overnight at 4°C in 2 volumes of the above Tris buffer containing Triton X-100 at a final concentration of 1%. The mixture was then centrifuged for 1 h at 100,000 x g in a Beckman ultracentrifuge and the recovered supernatant was stored at -70°C. The AChR in the Triton extracts was determined by measuring the amount of ^{125}I-α-BTX that coprecipitated with the receptor in ammonium sulfate at 35% saturation. Unbound toxin was removed by filtration through GF/C filters, and radioactivity retained on filters, i.e. toxin bound to receptor, was measured in a γ-counter.